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Title: Simultaneous Determination of Fluid Shifts during Thermal Stress in a Small Animal Model.

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Abbreviated Title: Fluid shifts in a small animal model

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER M39/85	2. GOVT ACCESSION NO. AD-A159363	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Simultaneous Determination of Fluid Shifts During Thermal Stress in a Small Animal Model		5. TYPE OF REPORT & PERIOD COVERED 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Michael J. Durkot, Ph.D., Osvaldo Martinez, Denise Brooks-McQuade and Ralph P. Francesconi, Ph.D		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Research Institute of Environmental Medicine Heat Research Division, Natick, MA 01760-5007		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3M161102BS10 54382101005
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE September 1985 13. NUMBER OF PAGES 18
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLAS 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

DISTRIBUTION A: Approved for public release; distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Body fluid compartments, dehydration, conscious rat model

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have developed methodology to measure simultaneously fluid re-distribution among the major compartments during moderate and severe hypohydration. Total body water (TBW) was determined using tritiated water, extracellular fluid volume (ECF) was measured using a single injection C^{-14} inulin, technique, and plasma volume (PV) was determined by cardio-green dye dilution. Moderate (10% decrease in body weight) and severe (15%) hypohydration resulted in significant losses in TBW, ECF and PV. Plasma volume was decreased by approximately 25% in

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both groups, and other fluid compartments were differentially affected. For example, the moderately dehydrated group maintained PV by shifting fluid from the ECF and ISF (interstitial fluid) compartment while preserving the ICF (intracellular fluid) conversely, the severely dehydrated group maintained PV by redistributing fluid from both the ECF and ICF compartments. The data indicated that the initial response to fluid loss was the movement of fluid from the ECF pool to sustain both PV and ICF. In severely hypohydrated rats, PV was maintained at the expense of ICF. These experiments indicated that PV and ICF were maximally protected, probably to preserve the integrity of the cardiovascular system and to minimize organ injury.

Abstract

We have developed methodology to measure simultaneously fluid redistribution among the major compartments during moderate and severe hypohydration. Total body water (TBW) was determined using tritiated water, extracellular fluid volume (ECF) was measured using a single injection C^{-14} inulin technique, and plasma volume (PV) was determined by cardio-green dye dilution. Moderate (10% decrease in body weight) and severe (15%) hypohydration resulted in significant losses in TBW, ECF and PV. Plasma volume was decreased by approximately 25% in both groups, and other fluid compartments were differentially affected. For example, the moderately dehydrated group maintained PV by shifting fluid from the ECF and ISF (interstitial fluid) compartment while preserving the ICF (intracellular fluid) conversely, the severely dehydrated group maintained PV by redistributing fluid from both the ECF and ICF compartments. The data indicated that the initial response to fluid loss was the movement of fluid from the ECF pool to sustain both PV and ICF. In severely hypohydrated rats, PV was maintained at the expense of ICF. These experiments indicated that PV and ICF were maximally protected, probably to preserve the integrity of the cardiovascular system and to minimize organ injury.

Key words: body fluid compartments, dehydration, conscious rat model

Introduction

Lee and Mulder (15) and Adolph (1) were among the first to report that when resting or moderately working man is exposed to heat stress without fluid replacement, progressive dehydration produces a disproportionately large reduction in plasma volume. While subsequent investigators have observed this response (5, 17, 21), discrepancies still persist with respect to the degree of plasma volume loss under various circumstances due to the various markers and methodologies used to measure plasma volume. For instance, Senay and Christensen (20) have questioned the validity of estimating plasma fluid shifts from dye-dilution techniques which are based on the stability of plasma proteins. Nevertheless, Harrison (12) monitored plasma albumin concentration and reported no significant vascular gain or loss of this protein during 2 hour of rest in the heat.

Unfortunately, the complexity of measuring fluid spaces is not limited to plasma volume measurement and is magnified when the intra- or extracellular (ECF) fluid compartments are assessed. Classical methods for determining extracellular volume utilize substances which 1) enter the tissue cells in a variable proportion (thiocyanate) (7), 2) are very rapidly excreted (sulfate) (22), or 3) are partially metabolized by the organism (mannitol)(8). Therefore, these methods may over-estimate the extracellular space. However, inulin (10) has several advantages over the aforementioned because it is not metabolized, stored, or incorporated by cells or erythrocytes, and it is not quickly excreted. However, the traditional use of inulin to measure ECV requires that the animal model be large enough to permit prolonged (6-8h) infusion to achieve an equilibrium concentration and also the infusion itself may appreciably change the ECF. Further, several samples must be taken to

monitor inulin decay, but only after the urinary bladder has been evacuated. Thus, this traditional methodology may compromise the results since volume re-adjustments probably occur during the infusion and the bladder evacuation requires anesthesia.

Recent studies on body fluid dynamics during exercise, heat acclimation or hypohydration have measured several fluid compartments under many experimental conditions which may affect the fluid pools. This diversity leads to discrepancies and inconsistent observations on extracellular fluid adjustments under these conditions. The purpose of the present investigation was to test the validity and reproduceablity of a ^{14}C-inulin, single bolus injection technique to measure extracellular space in a conscious, unrestrained rat model. This technique combines the positive features of the inulin methodology while eliminating the negative aspects of a long term infusion in an anesthetized model. Additionally, this technique was used to measure changes in this space induced by moderate and severe passive dehydration while simultaneously quantitating total body water and plasma volume shifts.

Materials and Methods

Male Sprague-Dawley rats (CD-1, Charles River Breeding Laboratories, Wilmington, MA) were purchased at 300 $\pm$ 20 g ($\bar{X} \pm$ SEM); thus, adequate time was available for acclimation to laboratory conditions before their targeted weight of 350g was achieved. All animals were caged individually in wire-bottomed cages in an environmental chamber (3x3x2 m) maintained at 26°C and 40% relative humidity with automatically time fluorescent lighting (on, 0600-1800 h). Rats were fed a standard diet (Ralston Purina Rodent Chow #5001) and water *ad libitum*.

Several days before an experiment, rats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg), and silastic catheters (.058 cm. ID) were surgically implanted in the right jugular vein and left carotid artery. The tips of the catheters were advanced approximately to the right atrium and arch of the aorta, respectively. The catheters were externalized through the dorsal surface of the neck, flushed with heparinized saline, and capped until use. The animals recovered from the effects of surgery for several days, and were judged to be healthy as evidenced by good appetite, normal weight gain, and general appearance.

When the animals had achieved the targeted experimental weight (350g), they were divided into three groups: control, moderate (10%), and severe (15%) hypohydration as determined by absolute weight loss. The animals were dehydrated to the appropriate level by passive heating (35°C) in a bacteriological incubator (Volard Electric Heating, Tarrytown, NY) for varying periods (4.5-11h). This temperature was selected because it elicited consistent states of dehydration in the two experimental groups without elevating the core temperature appreciably above 40.4°C, the threshold for heatstroke in men (16).

On the day prior to the experiment, animals were weighed, their core temperatures taken, and they were transferred to the incubator in individual cages without food and water. The incubator was wired to an automatic timer which activated the heating unit at the preset time(4.5h, 10% hyphydration and 11h, 15% hypohydration) and temperature (35°C). The control group was treated identically but the incubator temperature was maintained at 26°C, and the animals were allowed free access to water, but no food.

At approximately 0800h on the following day, the animals were removed from the incubator, re-weighed, core temperature taken, and returned to holding cages at 26°C. The arterial and venous lines were cleared to assure the unimpeded injection of markers and withdrawal of blood. Total body water (TBW) was determined by injecting $^{3}H_2O$ (New England Nuclear, Boston, MA) via the central venous catheter into the fully conscious rat. Approximately 800 nanocuries (nci) of $^{3}H_2O$ was administered in 200 ul of physiological saline. After equilibration (1.5h) one blood sample (.2 ml) was taken for the determination of total body water. Extracellular fluid volume (ECF) was then determined by injecting ^{14}C-Inulin (NEN, Boston, MA, 750 nci in 200 ul saline) via the central venous catheter. Arterial blood samples (.15ml) were taken at 2,4,6,30,60, and 90 minutes to determine decay characteristics. Plasma volume (PV) was measured using a tricarbocyanine dye (2mg in 1ml saline, Cardio-Green, Hyneon, Westcott and Dunniny, Division of Becton Dickinson Company) which has a peak spectral absorption at 800-810 nm in blood plasma. Arterial blood samples (200 ul) were taken at 3,5 and 7 minutes to determine the decay characteristics.

Analytic Methods

The heparinized blood samples were placed in ice immediately after withdrawal, centrifuged, and the plasma analyzed with minimal delay. Analysis of the plasma samples for $^{3}H_2O$ and ^{14}C-Inulin was performed with a Beckman LS8000 series liquid scintillation system using a standard library program for dual labels. Tricarbocyanine dye concentration was determined on a Hitachi Model 1200-60 Spectrophotometer (4). Core temperature was measured by Yellow Springs series 701 thermister probes inserted to a depth of 6cm.

Calculations

Total body water, extracellular fluid and plasma volume were calculated using the following equations:

1) Total Body Water (TBW, ml) (at Equilibrium 60 min) $= \frac{A\ (CPM)}{C\ (CPM/ml)}$

where A = total activity of $^{3}H_2O$ Injected (CPM)

C = activity of $^{3}H_2O$ in plasma (CPM/ml) at 60 min.

2) Extracellular Fluid Volume (ECF, ml) = $\frac{^{14}C\text{-Inulin injected (CPM)}}{*^{14}C\text{-Inulin plasma activity (CPM/ml)}}$

* theoretical plasma activity at T=0, which is Y intercept calculated from the non-linear double exponential regression equation where

$$Y = A \cdot \text{Exp}(BX) + C \cdot \text{Exp}(DX) + E$$

Determine Y = E (Intercept or activity at T=0, X = 0)

3) Plasma Volume (PV, ml) = $\frac{A\ (ug)}{C(ug/ml)}$

A=Total Dye injected (ug)

C=Theoretical plasma dye concentration at T=0

4) Intracellular Fluid Volume (ICF, ml)

ICF = TBW - ECF

5) Interstitial Fluid Volume (ISF, ml)

ISF = ECF - PV

Statistical differences between the means were calculated using a Hewlett Packard 9817 desktop computer, and the null hypothesis very rejected at $p<.05$ using Tukey's or Scheffe's test for significance.

Results

The effects of passive heating for either 4.5 h (moderate) or 11h (severe) at 35°C on body weight and core temperature are summarized in Table 1. Both experimental groups lost significant ($p<.05$) amounts of fluid as measured by absolute body weight changes. In addition, the rates of weight (fluid) loss were significantly ($p<.05$) increased in the moderately hypohydrated group,indicating greater water loss during the early hours. Passive heating for 4.5 and 11 h produced significantly ($p<.05$) elevated core temperatures in both groups. Since core temperatures in either experimental group rarely exceeded 40.4°C, the mortality figure was minimal despite the extreme level of dehydration.

Body fluid changes expressed as % of initial body weight or absolute volume (ml) are presented in Table 2. It is important to note that the control group exhibited normal values for all body fluid compartments (TBW, ECF and PV) (6,18,23). A 10% loss in total body mass (35.8g) was associated with a 4.38% ($p<.05$) decrease in TBW expressed as percent body weight or 24 ml decrease (9.6%) when expressed as absolute volume ($p<.05$). The 15% loss in total body mass (42.9g) was associated with a 6.9% ($p<.05$) decrease in TBW expressed as percent body weight and 37 ml decrease (14.8%) when expressed as absolute volume ($p<.05$). Both experimental groups lost significant ($p<.05$) ECF and PV when compared to the control group whether the data is expressed as % body weight loss or absolute volume (ml). However, when the two

experimental groups were compared to each other, only total body water showed significant ($p<.05$) differences.

The calculated values for intracellular fluid volume and interstitial fluid volume are presented in Table 3. The data indicate that severe hypohydration (15%) elicited a significant ($p<.05$) decrement in intracellular fluid while moderate dehydration (10%) effected a significant decrement ($p<.05$) in interstitial fluid when compared to controls. In addition there accrued a significant difference ($p<.05$) in intracellular fluid volume between the moderate and severe hypohydration groups.

Discussion

This study has provided a direct and simultaneous analysis of fluid volume redistribution during passive, heat-induced hypohydration in a small animal model. Although fluid compartments have been assessed under various physiological states (2,11,19), most investigators have used methodologies which measure a single compartment directly and other compartments by calculation and interpolation. Previously, Bauer et al (3) had made simultaneous determinations of fluid compartments; however, their methodology required a large anesthetized animal model, large sample volumes and complex analytical methodologies. The results of the present investigation indicate the reliability of a small conscious animal model to study such fluid shifts.

Interpretation of the current data requires consideration of several aspects of eating and drinking behavior in rodents. For example, rats are prandial drinkers; therefore, the presence of water in the absence of food does not assure proper hydration. In fact, it has been reported (19) that fasting animals for several days while allowing water ad libitum, elicited elevated hematocrits which increased with the time of food deprivation

indicating a progressive hypohydration. Another consideration is the normally high metabolic rate of these animals which causes an appreciable loss of water of oxidation and tissue mass with a simple overnight fast. Since it is difficult to separate weight loss from fluid loss in small animals, body weight changes may not totally reflect fluid loss especially when TBW is expressed as a percentage of body weight as opposed to absolute volumes. Our data are consistent with this observation; for example when water loss was expressed as a percentage of total body weight and compared to TBW loss measured directly by isotope techniques, a 10% loss of body weight was associated with only a 4.4% decrease in TBW. However if the percent weight loss due to an overnight fast (5.8%) and TBW loss (4.4%) as a per cent of body weight are considered, then close agreement is achieved. Similar observations were made for the severely dehydrated group. It should be noted that despite the 5.8% loss in body weight in controls, the total body water measurements made by isotopic dilution are identical to those of Culebras et al (6) who subsequently validated this methodology by total body dessication in the same animal. In addition, our control values for ECF and plasma volume agree closely with reported values determined by other markers or methodologies (13, 18, 23).

Our data indicate that passive heating elicited a re-distribution in body fluids designed to maintain plasma volume at a minimally reduced level in both moderate and severe hypohydration groups. Maintenance of plasma volume was apparently accomplished in the severely hypohydrated group by redistribution of intracellular fluid into the plasma compartment. Conversely, the moderately hypohydrated group apparently maintained plasma volume at the expense of interstitial fluid while maintaining intracellular fluid. Our

data are consistent with those of Horstman and Horvath (14) who have reported that the intracellular fluid volume is unaltered during moderate hypohydration. Thus it appears that the intensity of the fluid loss determines the degree of reliance on intracellular fluid to maintain plasma volume. Although Horowitz and Samueloff (13) found no statistical change in plasma volume during severe (16% body wt loss) dehydration, we believe that the disparate results may be explained by the methodologies used and the animal model (anesthetized and nephrectomized) employed.

We have concluded that the ability to maintain core temperature below 40.4°C, which is the theshold for heatstroke mortality, enabled the severely hypohydrated group to survive. It seems likely that since the animals were able to maintain plasma volume by shifting fluids into the plasma compartment, sufficient volume was maintained to assure heat dissipation and cardiovascular stability.

In summary, we have developed methodology to measure simultaneously fluid re-distribution in the major fluid compartments during passive heating dehydration. The data indicated that during heat-induced dehydration fluid is shifted to the vascular compartment to maintain plasma volume thereby defending cardiovascular and thermoregulatory mechanisms. When challenged by moderate dehydration this shift is accomplished by a decrease in ECF and ISF and maintainence of ICF. However, when the challenge intensifies, not only the ECV but also the ICF contribute to homeostasis. It appears likely that an increased hypohydration would compromise all compartments probably culminating in the multiple organ failure manifested in dehydration heatstroke.

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Disclaimer Statement

The views, opinions and findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other offical documentation.

Statement On Animal Use

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

Acknowledgements

The authors gratefully thank Larry Drolet for his valuable technical assistance and Susan Henry for manuscript preparation.

TABLE 1. BODY WEIGHT AND CORE TEMPERATURE CHANGES IN CONTROL, MODERATE AND SEVERELY HYPOHYDRATED RATS

	CONTROL[1]	MODERATE[2]	SEVERE[3]
PRE WT. (G)	358 ± 5.7	353 ± 9.5	350 ± 4.2
POST WT. (G)	337 ± 5.2	317 ± 8.4	307 ± 4.3
WT. LOSS (G)	20.1 ± .2	+35.8 ± .8×	+42.9 ± 1.4
% WT. LOSS	5.8 ± .2	+10.3 ± .3×	+14.3 ± .4
RATE WT. LOSS (MG/MIN)	20.8 ± 3	+58.5 ± 4×	+34.8 ± 5
INITIAL C.T. (°C)	37.7 ± .05	37.3 ± .09	37.6 ± .05
FINAL C.T. (°C)	37.5 ± .05	+39.4 ± .07×	+40.3 ± .11

RESULTS ARE GIVEN AS $\bar{X} \pm$ S.E.M.
[1]CONTROL: (OVERNIGHT FAST) WATER AD LIB., 26°C
[2]MODERATE: (OVERNIGHT FAST) NO WATER 4.5 HR., 35°C
[3]SEVERE: (OVERNIGHT FAST) NO WATER 11.5 HR., 35°C
+ SIGN. DIFFERENT FROM CONTROLS, $P < .05$
× SIGN. DIFFERENT BETWEEN MODERATE AND SEVERE $P < .05$

TABLE 2. TOTAL BODY WATER, EXTRACELLULAR FLUID VOLUME AND PLASMA VOLUME IN CONTROL, MODERATELY AND SEVERELY HYPOHYDRATED ANIMALS EXPRESSED AS PERCENT OF TOTAL BODY WEIGHT AND ABSOLUTE VOLUME.

	CONTROL[1]	MODERATE[2]	SEVERE[3]
TBW %	69.94 ± .47	65.56 ± .62 +	63.03 ± .39 +
ECF %	17.37 ± .74	13.09 ± .53 +	14.28 ± .60 +
P V %	4.48 ± .09	3.89 ± .06 +	3.81 ± .06 +
TBW (ML)	250 ± 4.2	226 ± 5.9 +	213 ± 3.3 +
ECF (ML)	61.87 ± 3.1	46.02 ± 1.9 +	49.24 ± 2.3 +
P V (ML)	17.67 ± .47	13.38 ± .27 +	12.90 ± .32 +

RESULTS ARE GIVEN AS $\bar{X} \pm$ S.E.M.

[1]CONTROL: (OVERNIGHT FAST) WATER AD LIB., 26°C

[2]MODERATE: (OVERNIGHT FAST) NO WATER 4.5 HR., 35°C

[3]SEVERE: (OVERNIGHT FAST) NO WATER 11.5 HR., 35°C

+ SIGN. DIFFERENT FROM CONTROLS, $P < .05$

× SIGN. DIFFERENT BETWEEN MODERATE AND SEVERE $P < .05$

TABLE 3. CALCULATED VALUES FOR INTRACELLULAR AND INTERSTITIAL FLUID VOLUMES

	CONTROL[1]	MODERATE[2]	SEVERE[3]
ICF (ML)	188.1 ±1.4	180.8 ±4.0 [×]	[+] 164.1 ±1.5
ISF (ML)	44.2 ±2.5	[+] 32.6 ±1.3	36.3 ±3.2

RESULTS ARE GIVEN AS $\bar{X}$ ±S.E.M.
[1]CONTROL: (OVERNIGHT FAST) WATER AD LIB., 26°C
[2]MODERATE: (OVERNIGHT FAST) NO WATER 4.5 HR., 35°C
[3]SEVERE: (OVERNIGHT FAST) NO WATER 11.5 HR., 35°C
+SIGN. DIFFERENT FROM CONTROLS, $P < .05$
×SIGN. DIFFERENT BETWEEN MODERATE AND SEVERE $P < .05$